Patients had a hematologic malignancy (ALL, AML, CML, MDS) and approximately 40% were in first complete remission or first chronic phase. 93% of transplantations used donors younger than 50 (median 35.8, range 18-61). The majority of patients received myeloablative preparative regimens (88%) and bone marrow grafts (62%). All patients received calcineurin inhibitor containing GVHD prophylaxis and 20% received *in vivo* T cell depletion. We identified three donor characteristics associated with overall survival; donor age, high resolution donor-recipient HLA-match and ABO blood group match. Risk adjusted 5-year survival rates were 37%, 33% and 29% with donors aged 18-32, 33-50, and >50 years, respectively ($p<0.0001$). Corresponding hazard ratios were 1.13 ($p=0.0004$) for donors aged 33−50 years and 1.29 ($p<0.001$) for donors >50 years compared with donors aged 18 − 32 years. Mortality risks were higher with one (HR 1.24, $P < .0001$) and two (HR 1.62, $P < .0001$) HLA-mismatches compared with HLA-matched transplants and minor (HR 1.10, $P = .002$) or major (HR 1.13, $P = .001$) ABO blood group mismatch compared with ABO-matched transplants. A subanalysis investigated the possibility that the association with donor age may be due to underlying genetic disparity between donor and recipient. That is, recipients with rare HLA genotypes tend to have fewer matched donors to choose from and thus more likely to receive a graft from an older donor. Recipient genotypes were assigned to quartiles using frequency data from 4 million NMDP donors as a reference. The lowest frequency quartile was associated with donors older than 50 ($p<0.0001$) and higher rates of HLA mismatching ($p<0.001$) but no association was found between genotype frequency and overall mortality. Acute GVHD risks were associated with donor age and HLA match and, donor parity, the only donor characteristic associated with chronic GVHD (table). In summary, the data recommend the consideration of donor age and ABO blood group match to maximize survival when selecting among comparably HLA-matched adult unrelated stem cell donors for treatment of a hematologic malignancy.

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Higher Infused CD34+ Dose Positively Influence Platelet Recovery After Cord Blood Transplantation

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Background: Umbilical cord blood transplantation (UCBT) is associated with delay in platelet recovery. However, the underlying factors influencing this delayed recovery remain poorly understood. With the aim of identifying factors that influence platelet engraftment, we retrospectively analyzed data from 68 consecutive myeloablative UCBT recipients transplanted between April 2006 and March 2012.
Methods: Forty-two (62%) patients received high-dose TBI (1320 cGy), cyclophosphamide and fludarabine (FLU); while 26 (38%) received Treosulfan, FLU, and a single fraction of 200cGy TBI. Graft-versus-host-disease (GVHD) prophylaxis consisted of cyclosporine and mycophenolate mofetil. Platelet recovery was defined as the first day of a platelet count ≥20 and ≥50 x 10^9/L without transfusion for 7 days. Cumulative incidence (CI) curves were used to estimate the probabilities of platelet engraftment in the first 100 days post- transplant. A proportional hazards regression model was used to evaluate the association between $CD34^+$ cell dose and platelet engraftment. Factors considered as potential predictors or confounders included age at transplant, sex, race, body mass index (BMI), disease risk, CMV seropositivity, presence of minimal residual disease at transplant (MRD), number of UCB units infused, acute GVHD, total nucleated cells (TNC) and $CD34^+$ cells.
Results: Median age and BMI were 36 years (range, 1-63) and 26 kg/m^2 (range, 16-41), respectively. The majority of patients (88%) received 2 cord blood units (n=60). Twenty-seven (40%) had MRD and 46 (68%) were CMV seropositive at time of transplant. Median total infused cell doses were: 3.9 x 10^7 TNC/kg (range: 2.2-11.2) and 0.25 x 10^6 $CD34^+$ cells/kg (range: 0.09 − 1.46). The 100-day CI of platelet engraftment was 65% (95% CI: 53-76%) for platelets ≥20 10^9/L and 59% (95% CI: 47-71%) for platelets ≥50 10^9/L. In univariate analysis higher $CD34^+$ infused dose was significantly associated with platelet engraftment [HR=1.4 (95% CI: 1.0-1.9, $P = .03$)]. Furthermore, platelet engraftment was suggestively slower among patients with higher BMI [HR=0.9 (95% CI: 0.9-1.0, $P = .06$)] while presence of aGVHD grade III-IV was associated with higher rate of engraftment [HR=2.1 (95% CI: 0.8-5.9, $P = .07$)]. No significant associations were found between all the others factors analyzed and platelet recovery.In multivariable analysis the association between larger $CD34^+$ dose and higher rate of engraftment remained statistically significant [HR=1.9 (95% CI: 1.3-2.8, $P < .001$]. In addition, CMV seropositivity [HR=0.4 (95% CI: .0.2-0.9, $P = .02$)] became significantly associated with a slower rate of engraftment.
Conclusions: These results indicate that the infused $CD34^+$ dose is a strong independent predictor of platelet engraftment. Furthermore we should expect an earlier sustained platelet recovery among CMV seronegative patients.

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Ultra-Low Dose IL-2 Expands Natural Regulatory T Cells and CD56bright NK Cells in Patients and Healthy Donors and Is Associated with Clinical Improvement in Chronic Graft Versus Host Disease

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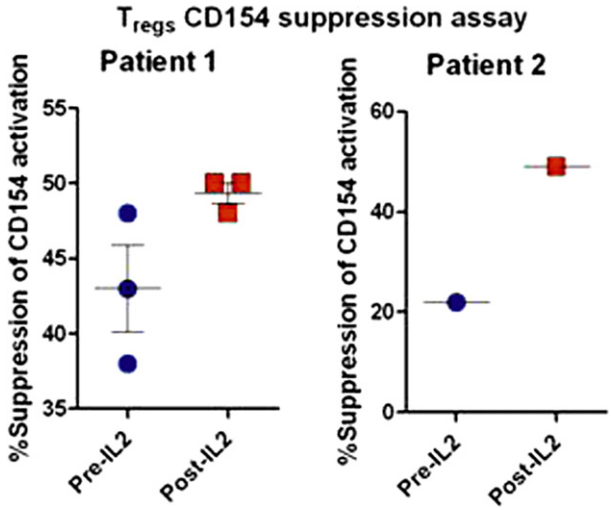
Low dose interleukin-2 (IL-2) can increase regulatory T cells (T_{reg}) and has therapeutic benefit in steroid refractory cGVHD. To further define immunological changes underlying the response to IL-2 and to determine tolerability, we performed phenotypic characterization and functional analysis of T_{reg} and natural killer (NK) cells in individuals given IL-2. Six healthy volunteers received subcutaneous ultra low dose IL-2 (0.2MIU/m^2/day) for 5 days without significant side effects. Two patients with steroid and calcineurin-refractory cGVHD received the same dose of IL-2 daily for 4 or 8 weeks, after HLA identical myeloablative stem cell transplant (SCT) for AML. Patient 1 (50 year old male, 3 yr post SCT) had skin and fascial GVHD affecting the lower limbs. Patient 2 (44 year old female, 2 yr post SCT) had upper gastrointestinal cGVHD. Both patients had a prompt and durable partial response by

NIH cGVHD criteria. Immunosuppression was subsequently withdrawn in patient 1 and reduced (budesdonide from 15 to 5mg) in patient 2. Mononuclear cells were analyzed by flow cytometry before and after IL-2 in patients and healthy donors. T_{reg} subsets were determined within the CD4+ T cell population to identify thymus-derived natural T_{reg} (nT_{reg}) and induced T_{reg} (iT_{reg}). NK cell subsets were determined within CD56+CD3- population to identify $CD56^{bright}$, $CD56^{dim}$NKG2A+KIR-, and $CD56^{dim}$ KIR+CD57+ NK cells. Functional analysis was performed by isolating T_{reg} ($CD3+CD4+CD25^{hi}CD127^{dim}$), conventional T cells (T_{con}: $CD3+CD4+CD25^{lo}CD127^{hi}$) and NK cells (CD3-CD56+). T_{reg} function was measured by suppression of CD154 expression on T_{con} stimulated with CD3/CD28 beads. NK cell function was measured by K562 killing. Healthy volunteers showed a significant increase in both nT_{reg} and iT_{reg} by 2-3 fold ($p=0.0003$) and $CD56^{bright}$ NK cells by 1.2-1.8 fold in 7 days after the initial dose of IL-2 ($p=0.02$). In patients, the fraction of nT_{reg} increased above baseline by 1.2-1.4 fold while iT_{reg} decreased. NK cells showed marked expansion of $CD56^{bright}$ NK cells within 2 weeks of treatment, with no change in $CD56^{dim}$NKG2A+KIR-, and $CD56^{dim}$KIR+CD57+ NK cells. Functional assays demonstrated increased T_{reg} suppression of autologous T_{con} and NK cell killing of K562 after IL-2 treatment in both patients. These findings indicate that ultra low dose IL-2 causes expansion of natural T_{reg} and NK cells resulting in greater suppressive activity and K562 killing activity correlating with an improvement in cGVHD for steroid refractory patients.



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Ex Vivo Treatment of Umbilical Cord Blood with Prostaglandin E2 Alters the Molecular and Functional Properties of T Cells Via Modulating Wnt/β-Catenin Signaling

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The favorable outcome of umbilical cord blood transplantation (UCBT) is compromised by prolonged time to engraftment, delayed immunologic reconstitution and late memory T cell skewing. Studies in zebrafish and mice have shown that the prostaglandin compound, 16,16 dimethyl prostaglandin E2 (PGE_2) increases HSC engraftment. We performed a Phase Ib clinical trial of double UCBT (dUCBT) using one untreated and one ex vivo PGE_2-treated UCB unit to determine safety and engraftment. 12 subjects with hematologic malignancies were enrolled. The PGE_2-UCB was the dominant source of hematopoiesis in 10 of 12 subjects with full T cell chimerism detected as early as 13 days. In UCB T cells, PGE_2 mediated increase of intracellular cAMP, activation of PKA and modulated the Wnt/β-catenin pathway as determined by upregulation of β-catenin and expression of Wnt target genes *Lef1*, *Tcf7* and Runx1. PGE_2 inhibited proliferation of UCB T cells in response to stimulation via CD3/CD28 and resulted in high expression of IL-7 receptor (CD127). Assessment of T cell reconstitution indicated that during the first year, the numbers of total T cells ($CD3^+$), $CD4^+$ and $CD8^+$ T subsets remained significantly lower ($p=0.036$) in PGE_2-UCBT recipients compared to dUCBT recipients without PGE_2. To examine whether PGE_2-mediated Wnt/β-catenin imprinting might be evident in PGE_2-UCBT recipients, we examined expression of the Wnt/β-catenin target Eomes, a transcription factor that links the long-term memory $CD8^+$ T cells to effector potency and protective immunity. Expression of Eomes was significantly elevated in PBMCs of PGE_2-UCBT recipients compared to controls. Consistent with the role of Wnt/β-catenin to maintain a central memory/stem cell memory $CD8^+$ phenotype, there was an increased fraction of $CD8^+CD62L^+$ cells in recipients of PGE_2-UCBT in contrast to the late memory T cell skewing in dUCBT recipients without PGE_2. Moreover, PGE_2-UCBT recipients displayed potent antiviral immunity resulting in a reduced incidence of CMV viremia and no EBV-mediated posttransplant lymphoproliferative disorder. Our findings indicate that PGE_2-UCB treatment induces Wnt-mediated gene programming and might favor the generation of long-lived memory $CD8^+$ T cells.

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Impaired B Cell Clonotype Diversification After Allogeneic Hematopoietic Cell Transplantation Predicts Graft-Versus-Host Disease

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Graft-versus-host disease (GVHD) is a detrimental complication of allogeneic hematopoietic cell transplantation (HCT) associated with impaired quality-of-life and decreased overall survival. Methods for predicting GVHD may enable use of pre-emptive therapeutic maneuvers to prevent or diminish clinical GVHD symptoms. Studies of GVHD in murine allograft models have shown that GVH reactions are associated with structural and functional derangements of lymphoid tissues, including lymph nodes, spleen, thymus, and bone marrow. Because these organs